ABSTRACT SUBMISSION FORM

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SEX + GENDER

Exploring the role of sex and gender on health research

CHRD
CHILD HEALTH RESEARCH DAYS

CHRD 2020: Abstract Submission Form

Submitter Name
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Title
De novo discovery of seven novel HYAL2 mutations in patients with cleft lip and /or palate cause HYAL2-deficiency: A biochemical insight

Background
Every three minutes, a child is born with a cleft lip or palate (CL/P) resulting in an incidence of 1 in 500-750 births. Mutations in HYAL2 encoding a cell-surface hyaluronidase have been reported as a novel cause of CL/P in humans. Recently, seven new variants in HYAL2 were identified in CL/P patients.

Objective
We hypothesize that these variants interfere with HYAL2 function making them the likely cause of CL/P in these patients.

Methods
To investigate if the HYAL2 variants altered HYAL2 expression and/or localization, each was introduced into the human HYAL2 cDNA and transiently transfected into HYAL2-deficient mouse embryonic fibroblasts. HYAL2 was detected by immunoblotting and the subcellular localization was evaluated by immunofluorescence. Cell surface localization of HYAL2 was verified with phosphoinositide-phospholipase C (PI-PLC), followed by immunoblotting. Immunoblots were quantified using luminescence units and normalized to a wild type control. The results were averaged and compared using a paired t-test.

Results
The levels of HYAL2 from the variants were evaluated from four independent transfections and compared to WT HYAL2. Variants-p.S65X and p.R295X resulted in no detectable HYAL2 protein. p.L238R, p.F425V and p.R277C showed significant reductions in detectable HYAL2 while p.G204A and p.H424Lfs*12 resulted in levels similar to that of the WT HYAL2. We observed very little cell-surface HYAL2 with immunofluorescence for p.L238R and p.G204A only. All the remaining variants showed no cell-surface HYAL2 but stable intracellular HYAL2 excluding p.S65X and p.R295X which showed a complete absence of stable HYAL2. PI-PLC released low levels of cell-surface HYAL2 only from p.L238R and p.G204A

validating the immunofluorescence results.

Conclusion
Stable expression levels and cell-surface localization of HYAL2 are crucial for its function. Our biochemical analysis confirms our hypothesis that all the HYAL2 variants interfere with its stability or localization. Therefore these variants are mutations and the likely cause of CL/P in the affected subjects.

Theme:
Basic Science

Do you have a table/figure to upload?
Yes

Untitled
Figures and Mutation nomenclature table.pdf

Are you willing to participate in Goodbear's Den?
Yes

Presenter Status:
Masters Student

What was your role in the project?
Perform Experiments

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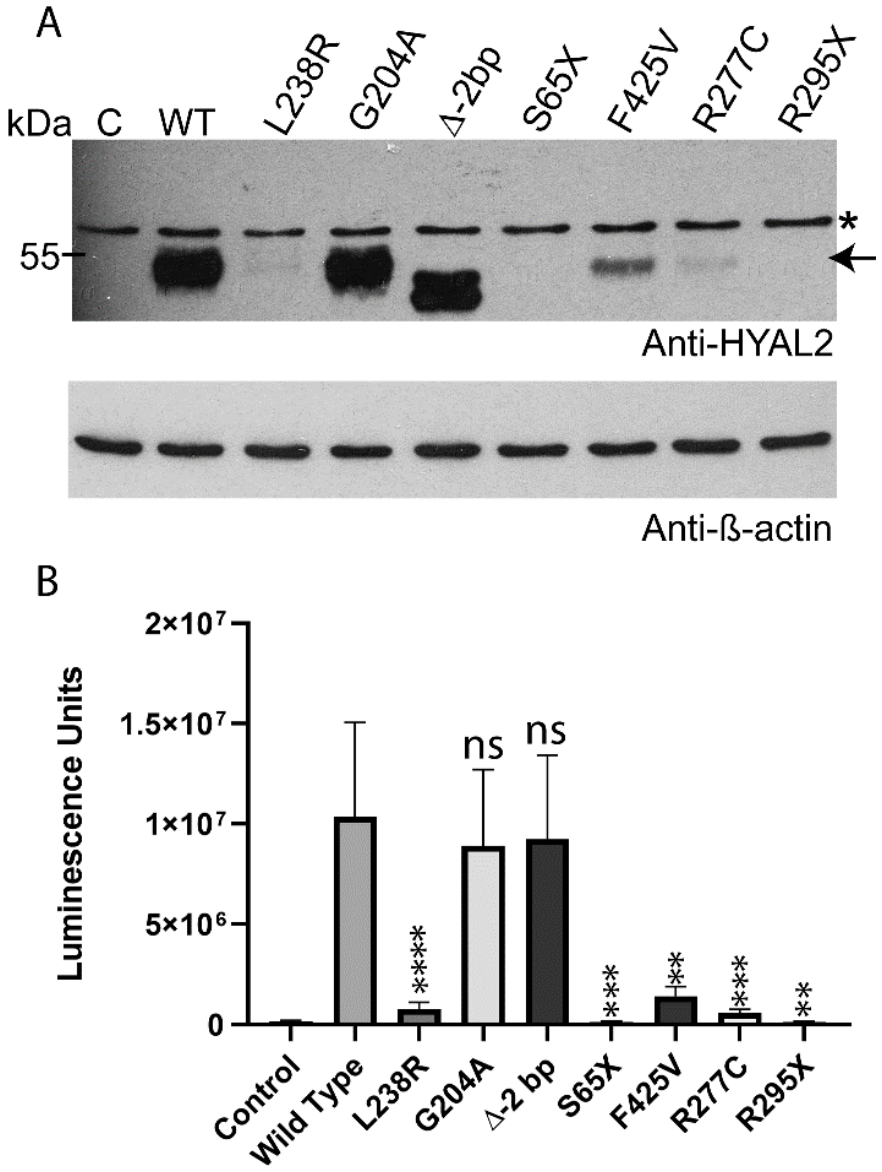

Figure 1. Expression of HYAL2 in *Hyal2-/-* mouse embryonic fibroblasts (MEFs). A. Immunoblot analysis of protein lysates (10 µg per lane) prepared from transfections with hHYAL2 expression vectors were analyzed with anti-HYAL2 (upper panel) and anti-β-actin (lower panel). HYAL2 is indicated by an arrow and the asterisk indicates a cross reacting band that is evident even in the control. The control was transfected with a vector that does not express HYAL2. B. Quantification of the immunoblots. The average ± SEM of the luminescence units from four separate transfection experiments are shown by the bars. The significance for each mutant compared to the WT was determined using a paired t-test using the ratios from each experiment. (ns-not significant; $p \leq 0.0001$ (****); $p \leq 0.001$ (***); $p \leq 0.01$ (**)

Please note: AC Del/-Δ2bp/ H424Lfs*12 have been used interchangeably throughout the document.

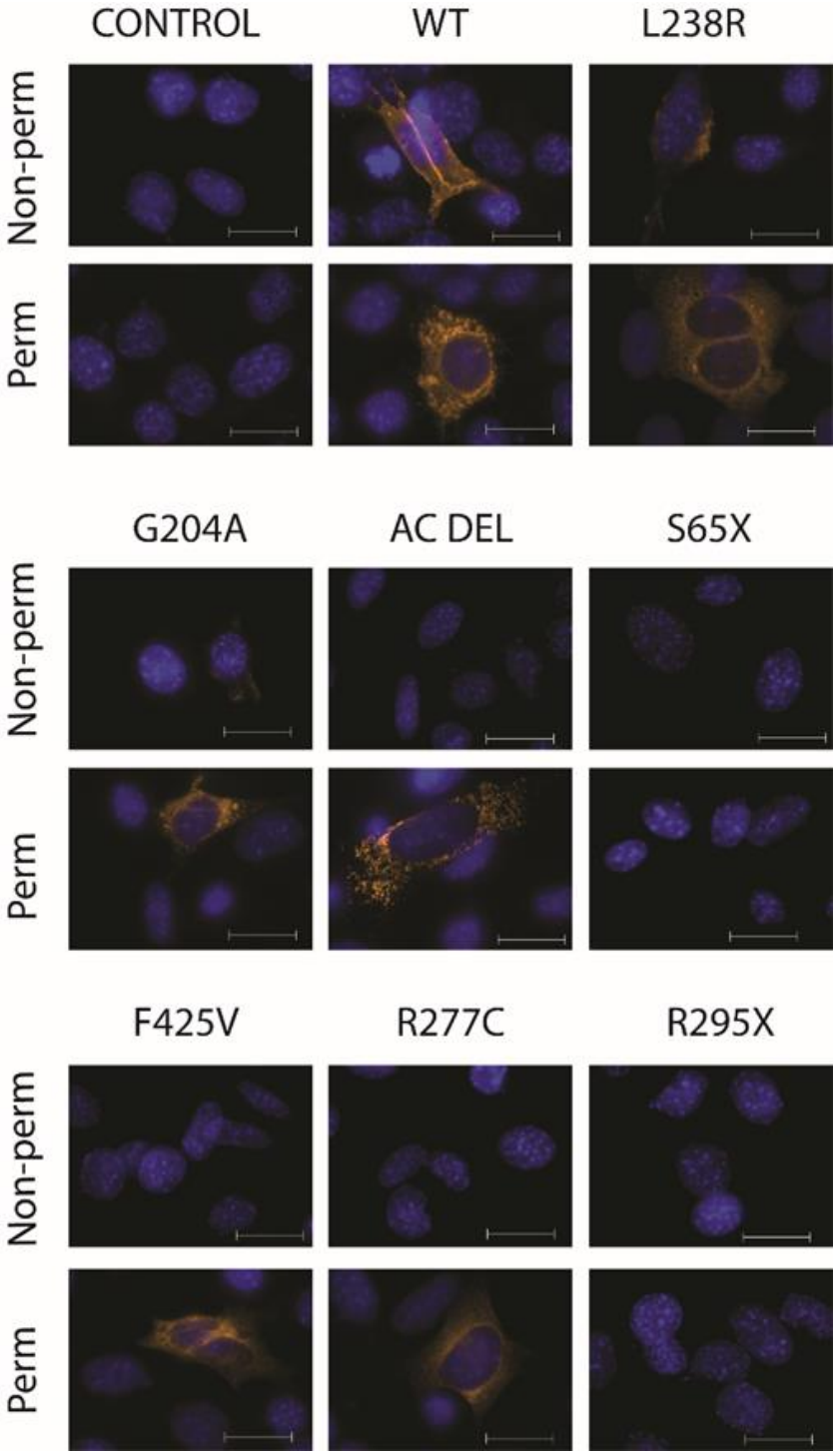

Figure 2. Comparative immunolocalization of *Hyal2-/-* MEFs expressing hHYAL2 variants. The transfected *Hyal2-/-* MEFs were fixed and incubated with anti-HYAL2 primary antibody under permeabilized and non-permeabilized conditions. hHYAL2 is detected with Alexa Fluor 568-conjugated donkey anti-rabbit secondary antibody and is labelled orange-fluorescent. Nuclei is stained blue with DAPI. Scale bars, 20µm. 63X magnification. Representative microscopy images of at least three independent experiments are shown.

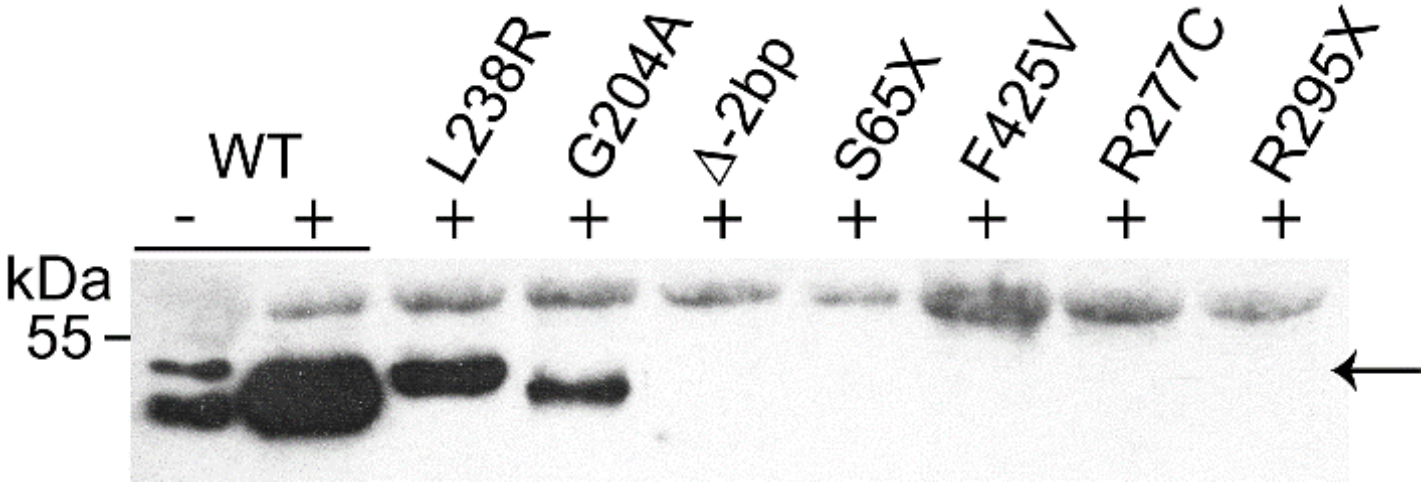

Figure 3. Immunoblot analysis of HYAL2 released by PI-PLC treatment. *Hyal2*$^{-/-}$ MEFs that were transfected with WT *HYAL2* and *HYAL2* variants were incubated with (+) or without (-) PLC to release the cell surface HYAL2. HYAL2 was detected by immunoblot after the protein in the media was concentrated.

MUTATION NOMENCLATURE

Mutation	Description
p.L238R	Leucine at position 238 replaced with Arginine
p.G204A	Glycine at position 204 replaced with Alanine
p.H424Lfs*12 (-Δ2bp/AC Del)	Frameshifting change with Histidine-424 replaced with a Leucine and creating a new reading frame at position 12; 2bp deletion results in the frameshifting and aa replacement
p.S65X	Serine at position 65 replaced with a stop codon (X)
p.F425V	Phenylalanine at position 425 replaced with Valine
p.R277C	Arginine at position 277 replaced with Cysteine
p.R295X	Arginine at position 295 replaced with stop codon (X)